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Research Article

# Description and phylogenetic position of a new species, *Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil (Saxifragaceae), from the southern islands of South Korea

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## Abstract

We describe a new species, *Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil, belonging to the family Saxifragaceae, from the southern islands of the Republic of Korea. *Chrysosplenium insularis* is morphologically similar to *C. japonicum* (Maxim.) Makino but can be distinguished by fairly persistent bulbils, green to yellowish-green sepals, four stamens, and cylindrical papillose seeds. *Chrysosplenium insularis* is also distinguished from *C. alternifolium* L., which is distributed in Europe, northern Russia, and the Caucasus, by the absence of stolons and green bracts. Phylogenetic analyses, based on one nuclear ribosomal (ITS) and two chloroplast (*rbc*L, *mat*K) regions, confirmed that the new species was monophyletic and that *C. insularis* and *C. alternifolium* formed a sister relationship with robust support. Herein, we provide a detailed morphological description of *C. insularis* with its corresponding geographical distribution and comparison table and figures of related species.

**Key words:** *Chrysosplenium*, morphology, new species, phylogeny, taxonomy

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## Introduction

*Chrysosplenium* L. is a perennial herbaceous genus of the family Saxifragaceae, consisting of more than 70 species (Kim et al. 2019; Fu et al. 2020). Species of this genus are mainly distributed in temperate regions of the Northern Hemisphere, and their habitats are shady and humid areas in the mountains (Kim and Kim 2015; Kim et al. 2018; Zhao et al. 2022). Biogeographically, *Chrysosplenium* is known to have originated in East Asia, and several independent lineages have migrated from East Asia to the New World (Soltis et al. 2001; Deng et al. 2015; Liu et al. 2016).

The genus *Chrysosplenium* is distinguished from other genera in Saxifragaceae by its tetramerous flowers with petaloid sepals and four or eight stamens (Kim et al. 2018). However, species delimitation is often difficult in this genus because of extensive morphological variations owing to differences in growth periods and habitats (Qin et al. 2018; Kim et al. 2019; Choi et al. 2020). The genus is divided into two sections, *Chrysosplenium* sect. *Alternifolia* Franch. and *C.* sect. *Oppositifolia* Franch., based on the arrangement of the leaves (Franchet 1890). However,

Hara (1957) proposed 17 series because of the high variability in the flower, capsule, and seed traits within each section. The infrageneric classification of *Chrysosplenium* species is based on several criteria, including leaf arrangement, seed surface, pedicel length, sterile branch position, capsule shape, stem surface, ovary position, stamen length, leaf surface, sepal length, and basal leaf size (Hara 1957; Fu et al. 2020). Several phylogenetic studies on Saxifragaceae genera, including *Chrysosplenium*, have been performed based on the chloroplast *mat*K region, and their results have shown that *C.* sect. *Oppositifolia* and *C.* sect. *Alternifolia* are monophyletic (Nakazawa et al. 1997; Soltis et al. 2001; Deng et al. 2015). Recently, several new species have been described based on detailed and comprehensive morphological, molecular, and cytological studies (Liu et al. 2016; Kim et al. 2018, 2019; Wakabayashi et al. 2018; Fu et al. 2020, 2021).

Thirteen *Chrysosplenium* species belonging to seven series have been recognized in the Korean Peninsula to date (Nakazawa et al. 1997; Kim et al. 2019; Choi et al. 2020; Korea National Arboretum 2021). The following are these 13 species [*Chrysosplenium* ser. *Pilosa* Maxim.: *C. flaviflorum* Ohwi, *C. epigealum* J.W.Han & S.H.Kang, *C. ramosissimum* Y.I.Kim & Y.D.Kim, *C. valdepilosum* (Ohwi) S.H.Kang & J.W.Han, *C. aureobracteatum* Y.I.Kim & Y.D.Kim, *C. barbatum* Nakai; *C.* ser. *Oppositifolia* Maxim.: *C. ramosum* Maxim.; *C.* ser. *Nepalensia* Maxim.: *C. grayanum* Maxim.; *C.* ser. *Sinica* Maxim.: *C. sinicum* Maxim.; *C.* ser. *Macrostemon* H. Hara: *C. macrostemon* Maxim. ex Franch. & Sav.; *C.* ser. *Alternifolia* Maxim.: *C. japonicum* (Maxim.) Makino, *C. serreanum* Hand.-Mazz.; *C.* ser. *Flagellifera* Maxim.: *C. flagelliferum* F.Schmidt], and among them, *C. aureobracteatum*, *C. barbatum*, *C. epigealum*, *C. flaviflorum*, and *C. ramosissimum* are endemic to Korea (Chung and Kim 1988; Korea National Arboretum 2021; Chung et al. 2023).

During a floristic survey in the southern part of Korea in March 2020, we found a new *Chrysosplenium* species that is restricted to the southern islands of Korea (Jeju-do and Gageo-do Islands). This species is readily distinguished from previously known *Chrysosplenium* species in Korea by its greenish-yellow to green bracteal leaves at flowering and a cylindrical papillose seed surface. This species is most similar to *C. japonicum* (Maxim.) Makino, which belongs to the *C.* ser. *Alternifolia*, and is distributed throughout Northeast Asia, including Southeast China, Japan, Korea, Russia (Manchuria), and Taiwan (Nakazawa et al. 1997; Pan and Ohba 2001; Hsu et al. 2011). The new species, however, is clearly distinguishable from *C. japonicum* by the form of bulbils, color of sepals, number of stamens, and surface of seeds. Based on thorough literature surveys, extensive field observations, detailed analysis of floral morphology and seed coat characteristics, we designated this new species as *C. insularis* J.E. Jang, K.H.Lee & H.Y.Gil. Here, we provide a detailed morphological description and phylogenetic position of *C. insularis* and its geographical distribution.

## Materials and methods

### Material collection

Field surveys were conducted from March 2020 to March 2023. Voucher specimens were deposited at the herbarium of the Korea National Arboretum (KH, http://www.nature.go.kr/kbi/plant/smpl/KBI_2001_030100.do). Materials preserved in 70% ethanol were used to observe and measure the floral parts. Mor-

phological observations and measurements of the new species were conducted on live and dried specimens, including the materials preserved at KH. Quantitative characteristics were measured based on at least 30 samples. The terminology used for description and comparison was referenced from Choi et al. (2020), Pan and Ohba (2001), Lozina (1939), Wakabayashi (2001), Kim et al. (2018), Kim et al. (2019), Fu et al. (2020), Fu et al. (2021).

## Microscopic observation

The seed morphology was observed under a stereomicroscope and a scanning electron microscope (SEM). The seeds were measured using a stereomicroscope (Carl Zeiss Microscopy GmbH, Stemi 508, Zeiss, Göttingen, Germany) with an Axiocam ERc 5s. Before SEM imaging, the seeds were dehydrated using 100% ethanol and sputter-coated with gold in a KIC-IA COXEM ion coater (COXEM Co., Ltd., Daejeon, Korea). SEM imaging was performed using a COXEM EM-30 PLUS+ table scanning electron microscope (COXEM) at 20 kV at the Seed Testing Laboratory of KH.

## Phylogenetic analysis

Molecular phylogenetic analyses were conducted to confirm the phylogenetic position of the new putative species of *Chrysosplenium*. Sixteen accessions of four taxa, including the new and related species, were collected from seven localities in South Korea. Total DNA was extracted from silica gel-dried leaves using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) in accordance with the manufacturer's instructions. The nrDNA region (ITS) and two cpDNA regions (*mat*K, *rbc*L) were subjected to polymerase chain reaction (PCR) (Choi et al. 2020) on a ProFlex 96-Well PCR System (Applied Biosystems, Foster City, CA, USA). The primers used and their sequences are listed in Table 1. Each reaction mixture contained AccuPower® PCR PreMix (Bioneer, Daejeon, South Korea), ca. 10 ng (1 µL) of genomic DNA, and 100 pM of primers in a total volume of 20 µL. The PCR conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of amplification at 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR products were visualized on 1% agarose gels and sequenced on an ABI 3730xl DNA analyzer using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences obtained were manually determined and aligned using MAFFT with Geneious Prime® 2022.1.1. (Biomatters Ltd., Auckland, NZ). The DNA sequences generated in this study have been deposited in GenBank and are indicated with an asterisk (*) in the voucher information in Table 2.

**Table 1.** Primers used for phylogenetic analysis.

| Fragment | Primer | Sequence 5' → 3' | Reference |
|---|---|---|---|
| ITS | ITS1 | TCCGTAGGTGAACCTGCGG | White et al. (1990) |
| | ITS4 | TCCTCCGCTTATTGATATGC | |
| *rbc*L | rbcL_1F | ATGTCACCACAAACAGAAAC | Fay et al. (1998) |
| | rbcL_724R | TCGCATGTACCTGCAGTAGC | |
| *mat*K | 3F_Kim_F | CGTACAGTACTTTTGTGTTTA | K.J.Kim, pers. comm. |
| | 1R_Kim_R | ACCCAGTCCATCTGGAAATCT | |

**Table 2.** Voucher information and GenBank number of accessions used in this study (*newly generated sequences).

| Taxon | Locality | Voucher information | GenBank number | | |
|---|---|---|---|---|---|
| | | | ITS | *rbc*L | *mat*K |
| *C. alternifolium* | JAPAN: Shimane-ken | *DG2019032310003* | OK315466 | OK315387 | OK315343 |
| *C. aureobracteatum* | KOREA: Gangwon-do, Mt. Gwangdeog | *LeeJD et al. 17127-1* | MK989508 | MK989534 | MK989559 |
| | KOREA: Gangwon-do, Mt. Gwangdeog | *LeeJD et al. 17127-2* | MK989509 | MK989533 | MK989562 |
| *C. barbatum* | KOREA: Jeollanam-do, Woldeung-myeon | *LeeJD et al. 17008-1* | MK989505 | MK989538 | MK989560 |
| | KOREA: Gyeongsangbuk-do, Mt. Danseok | *LeeJD et al. 17020-1* | MK989506 | MK989536 | MK989564 |
| | KOREA: Gangwon-do, Mt. Gwangdeog | *LeeJD et al. 17066* | MK989507 | MK989537 | MK989561 |
| *C. flagelliferum* | KOREA: Gyeongsangbuk-do, Ulleung-gun, Gwanmobong | *ESK21-267** | OR809214 | PP133187 | PP170153 |
| | KOREA: Gyeongsangbuk-do, Ulleung-gun, Gwanmobong | *ESK21-268** | OR809215 | PP133188 | PP170154 |
| | KOREA: Gyeongsangbuk-do, Ulleung-gun, Seonginbong | *AP22-025** | OR809213 | PP133186 | PP170152 |
| | KOREA: Gyeoggi-do, Mt. Cheonma | *LeeJD et al. 17014* | MK989499 | MK989530 | MK989585 |
| | KOREA: Gangwon-do, Mt. Cheongtae | *LeeJD et al. 17052-1* | MK989500 | MK989529 | MK989583 |
| | KOREA: Gyeongsangbuk-do, Ulleung-do | *LeeJD et al. 17122* | MK989501 | MK989531 | MK989584 |
| *C. flaviflorum* | KOREA: Gangwon-do, Mt. Pokkye | *ESK21-182** | OR809216 | PP133189 | PP170155 |
| | KOREA: Gangwon-do, Mt. Pokkye | *ESK21-183** | OR809217 | PP133190 | PP170156 |
| | KOREA: Gangwon-do, Mt. Pokkye | *ESK21-184-1** | OR809218 | PP133191 | PP170157 |
| | KOREA: Gangwon-do, Mt. Pokkye | *ESK21-184-2** | OR809219 | PP133192 | PP170158 |
| | KOREA: Chungcheongbuk-do, Mt. Gyemyeong | *LeeJD et al. 17030* | MK989513 | MK989542 | MK989569 |
| | KOREA: Gyeongsangbuk-do, Mt. Cheonglyang | *LeeJD et al. 17039* | MK989514 | MK989540 | MK989567 |
| | KOREA: Gangwon-do, Mt. Chiak | *LeeJD et al. 17048* | MK989515 | MK989541 | MK989568 |
| *C. grayanum* | JAPAN: Hokkaido, Sapporo, Mt. Maruyama | *Nakamura 16401* | MK989524 | MK989554 | MK989574 |
| | JAPAN: Hokkaido, Sapporo, Mt. Maruyama | *Nakamura 16402* | MK989523 | MK989553 | MK989575 |
| | JAPAN: Hyogo prefecture, Sasayama | *Lee JH & JS Shin s. n.* | MK989525 | MK989551 | MK989576 |
| | KOREA: Jeollanam-do, Mt. Cheongtae | *LeeJD et al. 17090-1* | MK989522 | MK989550 | MK989579 |
| | KOREA: Jeollanam-do, Mt. Cheongtae | *LeeJD et al. 17090-2* | MK989520 | MK989555 | MK989578 |
| | KOREA: Jeollanam-do, Mt. Cheongtae | *LeeJD et al. 17090-3* | MK989521 | MK989552 | MK989577 |
| *C. griffithii* | CHINA | *13PXD035* | MH809138 | MN185317 | MN451058 |
| *C.insularis* | KOREA: Jeju-do, Seogwipo-si, Hogeun-dong | *SOK-2022-175** | OR809225 | PP133198 | PP170164 |
| | KOREA: Jeju-do, Seogwipo-si, Hogeun-dong | *J.E.Jang et al. 230322** | OR809226 | PP133199 | PP170165 |
| | KOREA: Jeollanam-do, Gageodo | *K.H.Lee 230514-1** | OR809227 | PP133200 | PP170166 |
| | KOREA: Jeollanam-do, Gageodo | *K.H.Lee 230514-2** | OR809228 | PP133201 | PP170167 |
| *C. japonicum* | KOREA: Gyeonggi-do, Mt. Cheonma | *J.E.Jang 230325-1** | OR809220 | PP133193 | PP170159 |
| | KOREA: Gyeonggi-do, Mt. Cheonma | *J.E.Jang 230325-2** | OR809221 | PP133194 | PP170160 |
| | KOREA: Gangwon-do, Wonju-si | *S.R.Lee et al. 230420-1** | OR809222 | PP133195 | PP170161 |
| | KOREA: Gangwon-do, Wonju-si | *S.R.Lee et al. 230420-2** | OR809223 | PP133196 | PP170162 |
| | KOREA: Gangwon-do, Wonju-si | *S.R.Lee et al. 230420-3** | OR809224 | PP133197 | PP170163 |
| | KOREA: Jeollabuk-do, Mt. Chaegye | *LeeJD et al. 17022* | MK989502 | MK989548 | MK989586 |
| | KOREA: Chungcheongnam-do, Palbong-myeon | *LeeJD et al. 17025-1* | MK989504 | MK989549 | MK989587 |
| *C. kamtschaticum* | JAPAN: Hokkaido, Sapporo, Mt.Maruyama | *Nakamura 16403* | MK989516 | MK989539 | MK989566 |
| *C. ramosum* | KOREA: Chungcheongbuk-do, Daegang-myeon Goseong | *LeeJD et al. 17097-1* | MK989517 | MK989543 | MK989571 |
| | KOREA: Gangwon-do, Mt. Taegi | *LeeJD et al. 17147* | MK989518 | MK989545 | MK989573 |
| | KOREA: Gyeongsangbuk-do, Mt. Irwol | *LeeJD et al. 17205-1* | MK989519 | MK989544 | MK989572 |
| *C. sinicum* | KOREA: Jeju-do, Haean-dong | *LeeJD et al. 17043* | MK989528 | MK989557 | MK989582 |
| | KOREA: Gangwon-do, Mt. Cheongtae | *LeeJD et al. 17051-1* | MK989526 | MK989556 | MK989580 |
| | KOREA: Chungcheongbuk-do, Gagok-myeon | *LeeJD et al. 17086* | MK989527 | MK989558 | MK989581 |
| *C. valdepilosum* | KOREA: Gangwon-do, Mt. Taegi | *LeeJD et al. 17053-1* | MK989512 | MK989535 | MK989563 |
| | KOREA: Jeollabuk-do, Mt. Jiri | *LeeJD et al. 17057* | MK989510 | MK989532 | MK989565 |
| *Peltoboykinia tellimoides* | JAPAN: Nagano, Kiso-Fukushima, | *Okuyama 035251* | AB248847 | | |
| | CHINA: Zhejiang, Suichang County | *XXL170002-1* | | MZ779205 | |

We also included 32 accessions of 13 *Chrysosplenium* species deposited in GenBank and selected *Peltoboykinia tellimoides* (Maxim.) Hara as the outgroup (Soltis et al. 1996). A total of 47 accessions from 14 taxa were used for the phylogenetic analysis. Details of the voucher information and GenBank accession numbers of the species used in this study are provided in Table 2. Phylogenetic analyses were performed using the maximum likelihood (ML) method. For the ML analysis, the best-fit model was identified using ModelFinder in Phylosuite (Kalyaanamoorthy et al. 2017; Zhang et al. 2020). ML phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the GTR+ F + R3 model in the ITS regions and the TIM+F+R2 model in the combined chloroplast regions (Minh et al. 2013).

## Results and discussion

### Taxonomic treatment

***Chrysosplenium insularis*** **J.E.Jang, K.H.Lee & H.Y.Gil, sp. nov.**
urn:lsid:ipni.org:names:77350706-1
Figs 1, 4A–F

**Diagnosis.** *Chrysosplenium insularis* differs from *C. japonicum* in having fairly persistent bulbils, green to yellowish-green sepals, four stamens, and cylindrical papillose seeds.

**Type.** Korea • Jeju, Seogwipo-si, Hogeun-dong; 33.25084, 126.54434; elev. 58 m; 25 Mar 2020 [fl]; *Kang-Hyup Lee JJ-200325-001* [holotype KH (Fig. 2); isotypes, 3 sheets, KH].

**Description.** Perennial herbs, hermaphroditic, 5–15 cm tall. Bulbils present near stem base, fairly persistent, pink, turning darkish brown, pilose. Roots fibrous, white. Stems erect, cespitose, light green to green, sparsely hairy, without stolons. Basal leaves of flowering stems 1–6, opposite, simple, estipulate; petiole 3–9 cm long, glabrescent or sparsely hairy; blade reniform, 13–20 mm × 15–25 mm, apex rounded and often retuse, margins dentate to crenate, 13–17 teeth, base cordate, adaxially green, pilose, abaxially pale green, subglabrous. Cauline leaves of flowering stems 1–4, alternate, simple, estipulate; petiole 5–22 mm long, glabrescent or sparsely hairy; blade flabellate to reniform, 7–12 mm × 11–18 mm, apex retuse and often rounded or obtuse, margins dentate to crenate, 9–13 teeth, base cordate to broadly cuneate, adaxially green, pilose, abaxially pale green, subglabrous. Inflorescences terminal, 6–14 flowered cyme, surrounded by leaf-like bracts; peduncles 4.59–18.54 mm long; pedicels 0.5–1.5 mm long, sparsely pilose; bracteal leaves by inflorescence 3, petiole 0.2–4.7 mm long, glabrescent or sparsely hairy; blade subflabellate to orbicular, 2–18 × 2–14 mm, apex truncate and often retuse, margins dentate to crenate, 5–9 teeth, base broadly cuneate to subcordate, adaxially green, sparsely pilose to glabrescent, abaxially pale green, subglabrous. Flowers 4-merous, actinomorphic; sepals petaloid 4, free, erect to subspreading, ovate to broadly ovate, 1.2–2.1 × 1.5–3.1 mm, apex obtuse or rounded, yellowish green to green, glabrous; stamens 4; filaments narrow conical, 0.3–0.4 mm long; anther 0.2 mm long, yellow; pistil 2-carpellate, semi-inferior; ovary 1-locular; styles 2, free, erect, 0.2–0.3 mm long; stigma round; disc present. Capsules 2-lobed, horn shaped, lobes subequal, 2.8–3.7 × 3.8–5.2 mm long, green, glabrous, dehiscent

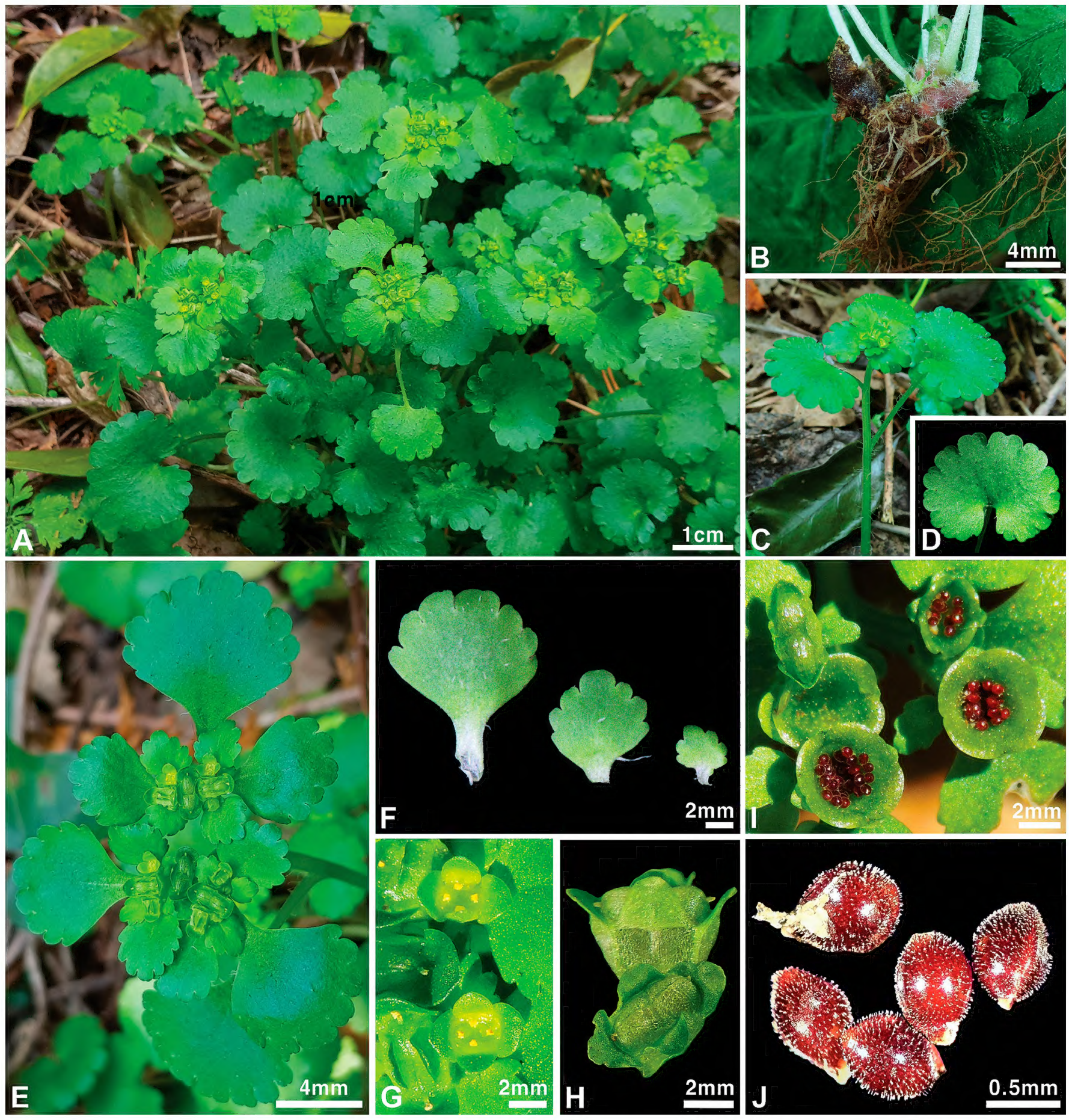


**Figure 1.** *Chrysosplenium insularis* **A** habit **B** bulbils **C** stem **D** basal leaf **E** inflorescence, **F** bracteal leaves **G** flower **H, I** capsule **J** seed. Photographs by Ju Eun Jang and Kang-Hyup Lee.

along the adaxial suture. Seeds numerous, ovoid-ellipsoid, 0.7–0.9 × 0.5–0.6 mm, brown to dark brown, cylindrical papillose on smooth surfaces.

**Phenology.** Flowering and fruiting from March to May.

**Distribution and habitat.** Southern coastal regions of Korea (Jeju-do and Ga-geo-do Islands). Forests, wet places in forests, shaded places on the riverside (Fig. 3).

**Etymology.** The specific epithet "*insularis*" refers to its distribution on islands.

**Vernacular name.** Island golden saxifrage: Seom-gwaeng-i-nun (섬괭이눈).

**Morphological assessment.** Among the species distributed in Korea, *Chrysosplenium insularis* is morphologically similar to *C. japonicum* in terms of leaf arrangement, leaf margin, and bracteal leaf color. Despite these similarities, it is clearly differentiated by the form of bulbils [present, fairly persistent (Fig. 1B) vs. present], surface of bracteal leaves [adaxially sparsely pilose to glabres-

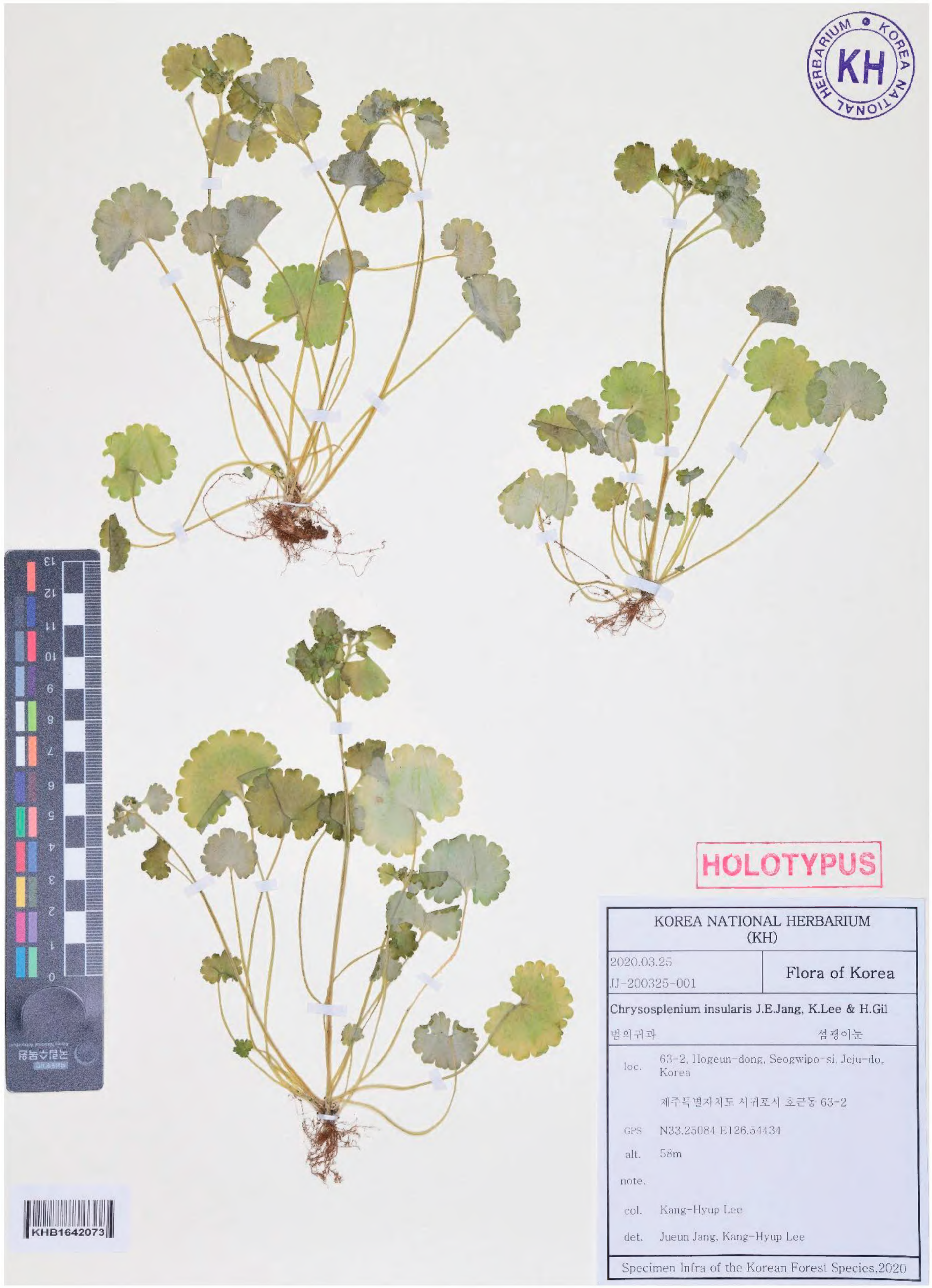


**Figure 2.** Holotype of *Chrysosplenium insularis*.

cent, abaxially subglabrous (Fig. 4D) vs. mainly glabrous (Fig. 4J)], color of sepals [green to yellowish green vs. yellowish green to yellow], number of stamens [4 vs. usually 8], and surface of seeds [cylindrical papillose (Fig. 4E, F) vs. papillose (Fig. 4K, L)]. Additionally, this new species is morphologically similar to *C. alternifolium*,

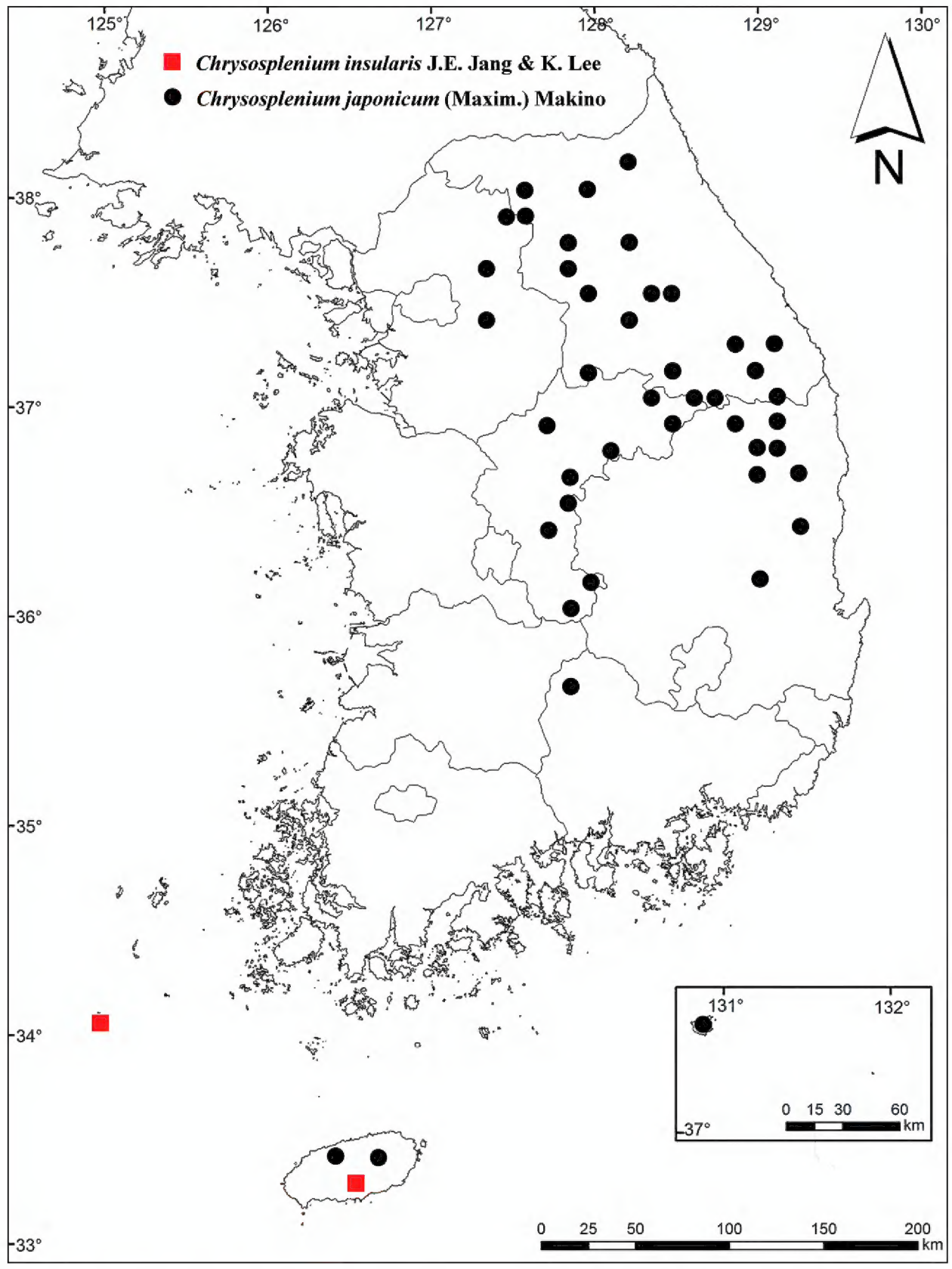


**Figure 3.** Distribution map of *Chrysosplenium insularis* and *C. japonicum* in Korea (revised from Oh et al. 2016).

which is distributed in northern Eurasia, but is distinguished by the following characteristics: stolon (absent vs. present), color of bracts (green vs. yellow), color of sepals (green to yellowish green vs. golden yellow), number of stamens (4 vs. 8), and surface of seeds [cylindrical papillose (Fig. 4E, K) vs. smooth (Fig. 4K, L)]. A comparison of the major characteristics of the new species with those of two closely related species, *C. japonicum* and *C. alternifolium*, is shown in Table 3.

**Phylogenetic analysis.** In total, 48 sequences of three regions (ITS, *mat*K, and *rbc*L) were newly obtained from the 16 accessions of *Chrysosplenium insularis* and the three related taxa. We also used 93 sequences from 32 accessions obtained from GenBank (12 species of *Chrysosplenium* and one *Peltoboykinia tellimoides* as an outgroup) for the phylogenetic analysis. The aligned matrix of the ITS region and combined chloroplast regions (*mat*K and *rbc*L) contained 635 and 1407 characters, respectively. We found 242 variable sites and 193 parsimony-informative sites in the ITS regions, whereas 172 variable sites and 104 parsimony-informative sites were found in the combined chloroplast regions. The GC ratios were 46.2% and 37.4% for the ITS and combined chloroplast regions, respectively. The phylogenetic tree (Fig. 5) revealed a topology similar to that obtained in a previ-

**Table 3.** Major characteristics of *Chrysosplenium insulalis* and two closely related taxa (*: data from Lozina 1939; -: none known).

| Character | | *C. insulalis* | *C. japonicum* | *C. alternifolia** |
|---|---|---|---|---|
| Bulbils | | present, fairly persistent | present | - |
| | color | pink, turning to darkish brown | pink | - |
| Stolon | | absent | absent | present |
| Bracteal leaves | color | green | yellowish green | yellow |
| | surfaces | adaxially sparsely pilose to glabrescent, abaxially subglabrous | mainly glabrous | mainly glabrous |
| Sepals | color | green to yellowish green | yellowish green to yellow | golden yellow |
| Stamens | number | 4 | usually 8 | 8 |
| Seeds | surfaces | cylindrical papillose | papillose | smooth |
| Fl. and fr. | | Mar. to May | Apr. to Jun. | Apr. to Jul. |

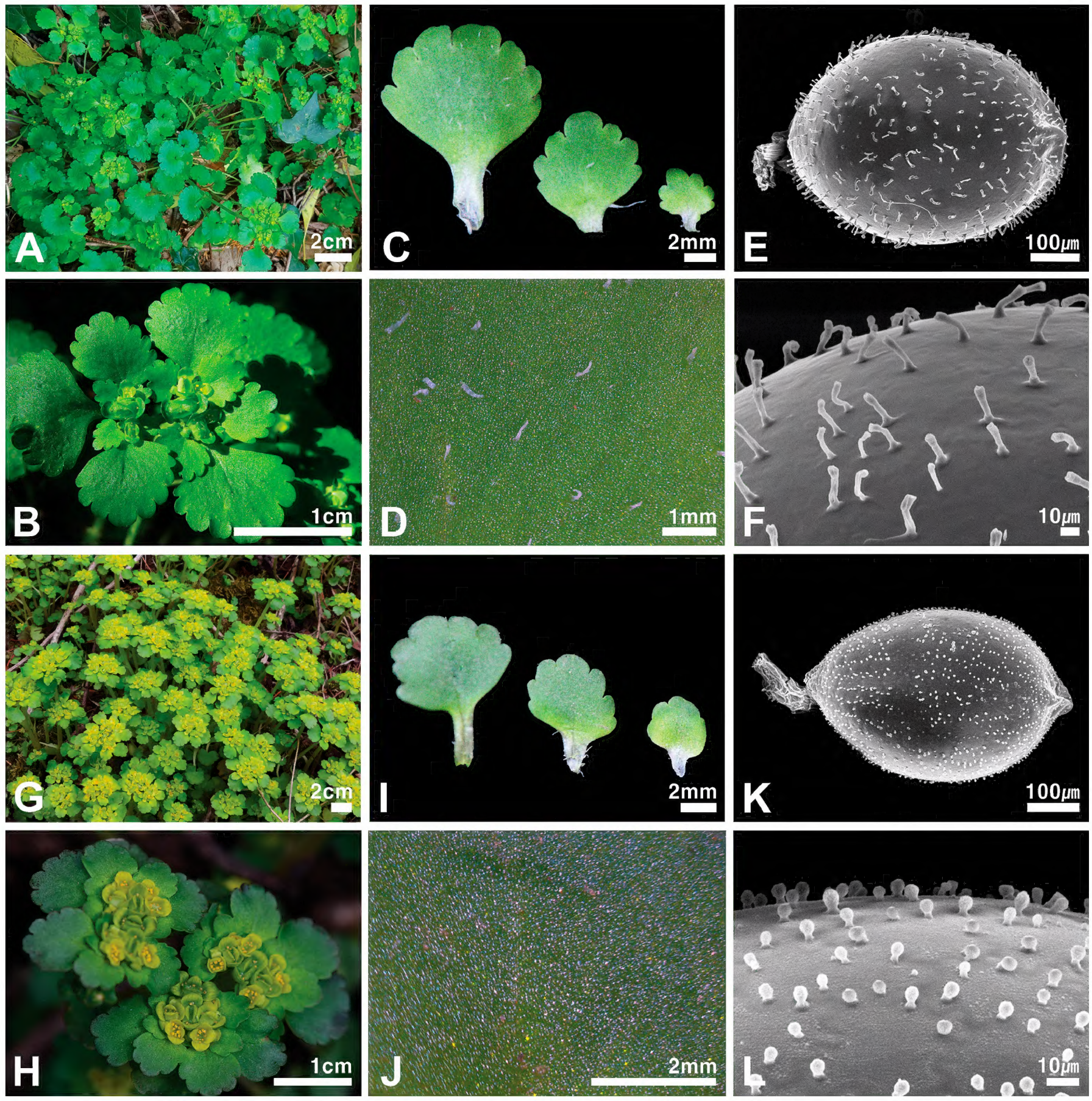


**Figure 4.** Comparative photographs of the habit (**A, G**), inflorescence (**B, H**), bracteal leaves (**C, I**), surface of bracteal leaves (**D, J**), and seed (**E, F, K, L**) of *Chrysosplenium insularis* (**A–F**) and *C. japonicum* (**G–L**). Photographs by Ju Eun Jang and Kang-Hyup Lee.

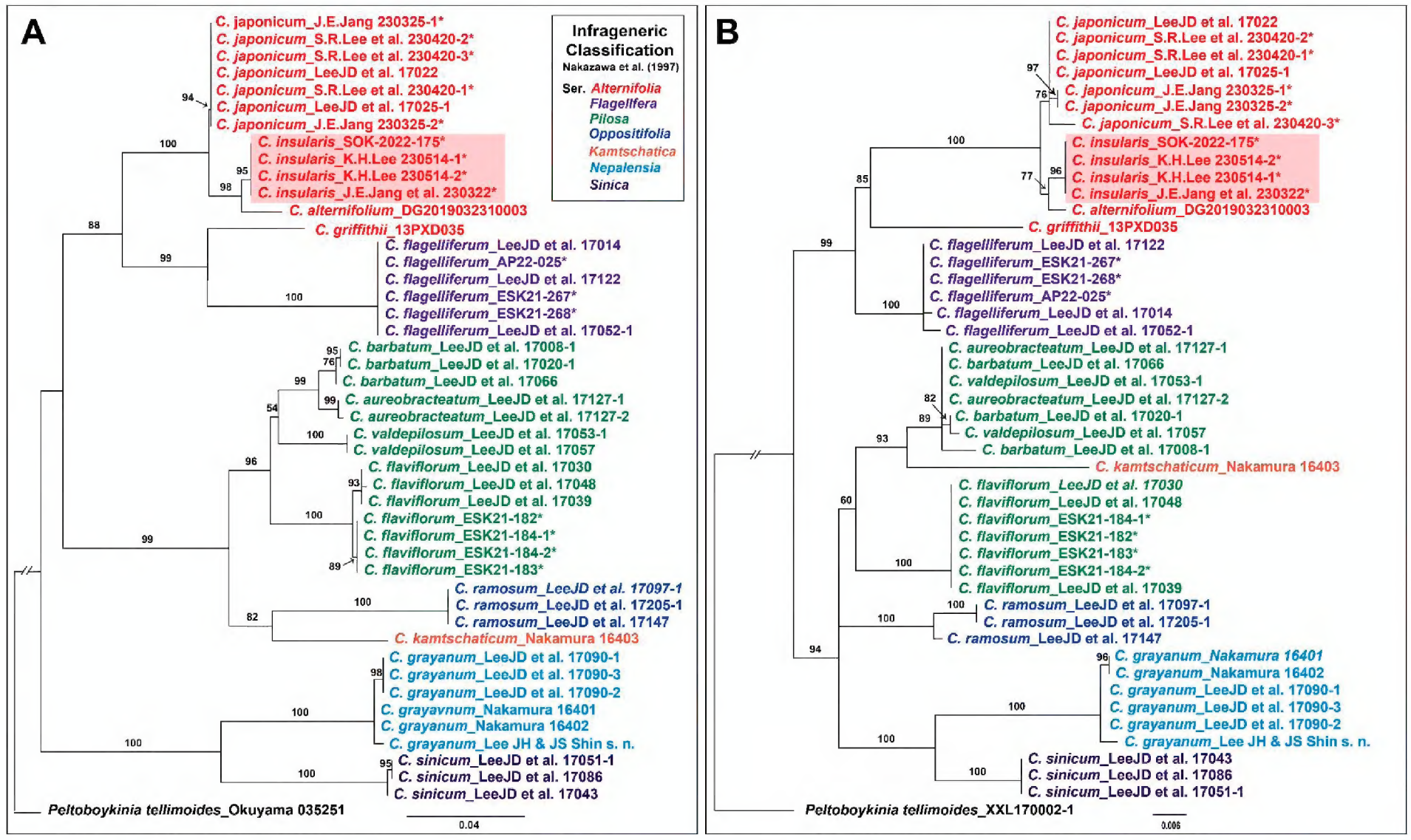


**Figure 5.** Phylogenetic tree of *Chrysosplenium insularis* and related taxa based on ITS regions and combined CP regions (*mat*K and *rbc*L) **A** ITS region **B** combined CP regions (*mat*K and *rbc*L). The numbers above the branches are bootstrap values (BS > 50%) by the maximum likelyhood method. Newly generated sequences in this study are shown with an asterisk, and the new species are marked with a red box. The voucher information of all samples used in the analysis is indicated after the scientific names.

ous study (Choi et al. 2020). The phylogenetic results showed some topological incongruence between the ITS and combined CP trees. In the ITS tree, the most basal clade (BS = 100%) included the monophyletic *C. grayanum* and *C. sinicum* and showed a sister relationship with other *Chrysosplenium* species. However, the CP tree was divided into two clades, with *C. grayanum* and *C. sinicum* sharing the most common ancestors with the *C.* ser. *Pilosa*, *C. kamtschaticum*, and *C. ramosum* (BS = 94%). The phylogenetic relationships among the three subclades were not fully resolved, and the *C.* ser. *Pilosa*. was not monophyletic, embedding *C. kamtschaticum*. Furthermore, the series *Alternifolia* was monophyletic in the CP tree but not in the ITS tree. Both trees strongly supported the monophyly of *C. insularis* (BS = 95% in ITS, BS = 96% in CP), and it shared the most common ancestor with *C. alternifolium* distributed in Japan (BS = 98% in ITS, BS = 77% in CP). The phylogenetic trees revealed that *C. insularis* formed an independent monophyletic clade from closely related taxa (i.e., *C. japonicum* and *C. alternifolium*), suggesting the newly recognized species of *Chrysosplenium* (Fig. 5).

**Additional specimens examined.** *Chrysosplenium insularis* (*Paratypes*): Korea • Jeonnam, Sinan-gun, Heuksan-myeon, Gageodo-ri; 14 May 2023; *K.H.Lee 230514-1* (KH). • Jeju, Seogwipo-si, Hogeun-dong; 28 Apr. 2020; *PBK0118-001* (KH). • Jeju, Seogwipo-si, Hogeun-dong; 22 Mar. 2022; *Hanon-220322-011* (KH) • Jeju, Seogwipo-si, Hogeun-dong; 22 Mar. 2023; *J.E.Jang et al. 230322-1* (KH).

*Chrysosplenium japonicum*: Korea • Gyeonggi, Gwangju-si, Chowol-eup, Mugap-ri, Mugapsan; 24 Apr. 2007; *HNHM-A-158* • Gwangju-si, Toechon-myeon, Cheonjinam; 7 Apr. 2000; *KNAH014041* • Gwangju-si, Toechon-myeon, Usan-ri,

Aengjabong; 11 Apr. 2004; *kjs040141* (KH) • Incheon-si, Ongjin-gun, Jawoldo Isl.; 8 Apr. 2009; *NAPI-2009-1214* (KH) • Incheon-si, Ongjin-gun, Deokjeok-myeon, Mungap-ri, Gitdaebong; 9 Apr. 2014; *Park140230* (KH) • Incheon-si, Ganghwa-gun, Ganghwado Isl.; 20 Apr. 2006; *LeeGH6-35* (KH) • Gyeonggi, Namyangju-si, Onam-eup, Cheonmasan; 17 Apr. 2009; *ParkSH90273* (KH) • Gyeonggi, Namyangju-si, Onam-eup, Palhyeon-ri; 25 May 2023; *J.E.Jang 230325-1* (KH) • Gyeonggi, Namyangju-si, Joan-myeon, Ungilsan; 11 Apr. 2009; *Y.M.Kang s.n.* (KH) • Gyeonggi, Gwacheon-si, Makgye-dong, Cheonggyesan; 8 Apr. 2006; *KHUS20110475* (KH) • Gangwon, Pyeongchang-gun, Yongpyeong-myeon, Jaesan-ri, Geumdangsan; 17 Apr. 2012; *JSY120434* (KH) • Jeongseon-gun, Imgye-myeon; 23 Apr. 2011; *0307013* (KH) • Gangwon, Taebaek-si, Hasami-dong, Deokhangsan; 23 Apr. 2005; *kjs050052* (KH) • Gangwon, Wonju-si, Panbu-myeon, Geumdae-ri; 20 Apr. 2023; *S.R.Lee et al. 230420-1* (KH) • Chungbuk, Danyang-gun, Danyang-eup, Suchon-ri, Sobaeksan; 17 Apr. 2005; *Sobaeksan-050417-070* (KH) • Chungbuk, Chungju-si, Sotae-myeon, Boktan-ri; 12 Apr. 2012; *Namhan-548* (KH) • Gyeongbuk, Gunui-gun, Bugye-myeon, Dongsan-ri, Palgongsan; 22 Apr. 2006; *CBU-070308* (KH) • Gyeongbuk, Bonghwa-gun, Myeongho-myeon, Bugok-ri, Cheongnyangsan; 27 Mar. 2006; *CBU-070519* (KH) • Chungbuk, Cheongsong-gun, Hyeonseo-myeon, Bohyeonsan; 22 Apr. 2006; *K.O.Yoo s.n.* (KH) • Jeonbuk, Namwon-si, Ayeong-myeon, Gusang-ri, Bonghwasan; 1 May 2007; *HNHM-A-283* (KH).

## Key to the species of *Chrysosplenium* in South Korea modified from Choi et al. (2020)

1a Cauline leaves alternate ..... **2**
2a Leaves heterophyllous; sterile branches developed; caluline and bracteal leaves 2–5 lobed ..... ***C. flagelliferum***
2b Leaves isophyllous; sterile branch absent; cauline and bracteal leaves not lobed with 8–12 teeth ..... **3**
3a Sepals green; stamens 4 ..... ***C. insularis***
3b Sepals yellowish green or golden yellow; stamens 8 ..... **4**
4a Stolons present; sepals golden yellow; seed surface smooth ..... ***C. alternifolium***
4b Stolons absent; sepals yellowish green; seed surface papillose ..... ***C. japonicum***
1b Cauline leaves opposite ..... **5**
5a Sepals green, spreading; capsules cup-shaped ..... ***C. ramosum***
5b Sepals yellow, erect; capsules horn-shaped ..... **6**
6a Plants glabrous ..... **7**
7a Stamens 4 (-6); cylindrical papillae with roundish head at the tip on smooth seed ..... ***C. grayanum***
7b Stamens 8; cylindrical papillae with truncate tip on scabrous seed surfaces ..... **8**
8a Sterile branches present; plant glabrous except petiole of sterile branches; stamens shorter than the sepals ..... ***C. sinicum***
8b Sterile branches absent; plant glabrous; stamens longer than the sepals ..... ***C. macrostemon***
6b Plants pubescent ..... **9**
9a Seeds without tubercules ..... **10**

10a Leaves of sterile branches congested at the distal end, with white variegated veins on the upper surface ..... ***C. flaviflorum***
10b Leaves of sterile branches distantly arranged, with silvery dotted upper surface..... ***C. epigealum***
9b Seeds with tubercules..... **11**
11a Seed tubercles arranged on inconspicuous longitudinal ridges ..... **12**
12a Sterile branches highly branched, ca. 30 cm long after fruiting; leaves of sterile branches with silvery dots, upper surface glabrous; bracteal leaves yellowish-green..... ***C. ramosissimum***
12b Sterile branches unbranched, less than 15 cm long after fruiting; leaves of sterile branches without silvery dots, upper surface pilose; bracteal leaves bright yellow..... ***C. valdepilosum***
11b Seed tubercles arranged on prominent longitudinal ridges..... **13**
13a Leaves of sterile branches distantly arranged after fruiting; bracteal leaves golden yellow, greenish yellow at flowering..... ***C. aureobracteatum***
13b Leaves of sterile branches congested at the distal end after fruiting; bracteal leaves green at flowering..... ***C. barbatum***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

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### Data availability

All of the data that support the findings of this study are available in the main text.

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